

Comparative Estimation of Arginine Content in Peanuts: An Analytical Study

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ABSTRACT

Introduction: Arginine is an amino acid that has several positive effects on human health and peanut seeds are the richest source of it. Peanuts are the store houses loaded with proteins, fatty acids, vitamins, fiber and other phytochemicals. It has been shown that the intake of arginine at the concentration of 3-6 g/day has been found to improve the cardiovascular system, reduce intestinal permeability and activate the immune system that aids in early recovery of tuberculosis patients.

Aim: To evaluate the variation of arginine content in raw, boiled, soaked, soaked and boiled type of peeled and unpeeled peanuts

Materials and Methods: The analytical study on various groups of peanuts (raw, boiled, soaked, soaked and boiled peanuts of both peeled and unpeeled varieties) was conducted at Gayatri Vidya Parishad Institute of Health Care and Medical Technology at Visakhapatnam, Andhra Pradesh, India, in December 2019. The arginine content was estimated in peanut seeds using the Sakaguchi method by Spectrophotometry at wavelength of 520 nm. Descriptive statistics were done for all 12 groups and

were represented with mean, standard deviation and standard error. Analysis of Variance (ANOVA), Post-hoc Tukey's tests were applied to find statistical significance. The p-value <0.05 was considered as statistically significant. Statistical Package for Social Sciences (SPSS) version 25.0 was used for analysis.

Results: Concentration of arginine was recorded for each type in triplicate considering the mean. The raw soaked peeled peanuts had the lowest concentration of arginine (31.82 µg/g) and the soaked boiled for 15 minutes, unpeeled peanuts had the highest amount of arginine (1438.18 µg/g). Significant difference was found among the twelve groups by using ANOVA test p<0.001. Soaked and boiled 15 min peeled peanuts, soaked unpeeled, soaked and boiled 15 min unpeeled and soaked and boiled 30 min unpeeled were significantly different when compared to the remaining groups using Post-hoc Tukey's test p<0.05.

Conclusion: Soaked, boiled and unpeeled peanuts were the best types with the highest amounts of arginine which could be chosen as adjuvant nutritional therapeutic supplement to aid in the recovery of certain diseases.

Keywords: Adjuvant therapy spectrophotometry, Amino acids, Protein

INTRODUCTION

Arachis hypogaea Leguminosae is the botanical name of peanut, a legume crop that is grown mostly in the fields of tropics and semi-arid tropics [1]. This seed is an excellent repository of proteins, fatty acids, vitamins, fiber, and other phytochemicals [2]. Peanuts have high concentrations of arginine content, that is a non essential amino acid with numerous health benefits than many other nut crops given as a nutritional adjuvant therapy in patients with pulmonary tuberculosis. Intake of arginine at the concentration of 3-6 g/day has been found to improve the cardiovascular system, reduce intestinal permeability and activate the immune system [3,4]. Arginine being an amino acid forms the structural component of many proteins. Arginine and its role in human health have gained its recognition as a potential therapeutic agent that has a marked effect on cardiovascular disease [5], wound healing [6] and cancer [7-9]. Out of the 20 amino acids that are coding for a part of ribosomal protein synthesis in humans, arginine (2-amino-5-guanidinovaleric acid) is one of the compounds.

Many biological processes require arginine, which is degraded into chemical intermediates that reload the Krebs's cycle [10]. Arginine plays a key role in humans that functions as an intermediate of the urea cycle. In the metabolic pathway of the urea cycle, arginine acts as a carrier of nitrogenous waste. In that pathway, the final step is catalysed by the enzyme Arginase (ARG), that converts arginine to ornithine and urea, this allows urea to be available for excretion and regenerates ornithine to enter again into the cycle [11]. Though not well acknowledged clinically, the chemical equilibrium in the human body by acid/base balance is taken care of by arginine. Arginine is essential for T-cell proliferation [12-14]. Arginine plays a key role in host immune response acting as a substrate for nitric

oxide synthesis. This amino acid also functions as an important component for collagen synthesis, that aids in wound healing of mammals [15,16]. An additional function of arginine is its role in Nitric Oxide (NO) synthesis by vascular endothelial cells that regulate the tone of the vessels and function of cardiovascular system [17].

The human body contains enzymes that can synthesise arginine endogenously and therefore it is a non essential amino acid that does not need dietary supplementation [18]. However, arginine need is elevated during times of stress and growth, which makes dietary arginine intake mandatory. Hence, this amino acid arginine is classified as a conditionally essential amino acid [19]. The availability of literature regarding the use of arginine-containing nuts and seeds is very minimal. By supplementing the nutritional adjuvant, there might be an early recovery of individuals with cardiovascular disorders, lung infections and cancer patients. The present study aimed to evaluate the variation of arginine content in raw, boiled, soaked, types of peeled and unpeeled peanuts.

MATERIALS AND METHODS

This analytical study was conducted at the Gayatri Vidya Parishad Institute of Health Care and Medical Technology, Visakhapatnam, in the month of December 2019. Institutional Ethics Committee approval was obtained GVPIHCMT/IEC/20201208/04. The peanuts were purchased from a local market that was certified by Indian organic and NPOP/NAB/0017. This pack was approved by Food Safety and Standards Authority of India (FSSAI).

Inclusion criteria: Peanuts graded as special grade as per the grade designations in schedule II (Grade designations and definition of quality of Hand Picked Selected (HPS) Groundnut pods Commercially known as peanuts) published in Gazette

of India Part II, section 3 (ii) under S.O 2294 dated 26.6.1982 [20] (AGMARK).

Exclusion criteria: Damaged, broken kernel and nooks with any sign of white or green powdery mass were excluded from the study.

Study Procedure

Arginine estimation was done using the Sakaguchi method by Spectrophotometry at a wavelength of 520 nm [21]. The study variables were grouped as G1 to G12 based on the form of peanut that was used to estimate the arginine content. Boiling was done for 15 minutes or 30 minutes. Few other forms of peanuts were soaked overnight. The grouping was done as-

G1: raw peeled

G2: 15 minutes boiled peeled

G3: peeled boiled 30 minutes

G4: soaked peeled

G5: soaked and boiled 15 minutes

peeled

G6: soaked and boiled 30 minutes peeled **G7:** raw unpeeled

G8: boiled 15 minutes unpeeled

G9: boiled 30 minutes unpeeled

G10: soaked unpeeled

G11: soaked and boiled 15 minutes

unpeeled

G12: soaked and boiled 30 minutes unpeeled.

An amount of 150 g seed of each variety selected for arginine estimation was taken and using a blender, seeds were ground, and a 10 g sub-sample was further powdered using a mortar and a pestle. The sub-samples were then extracted using 30 mL of distilled water and filtered using no. 1 Whatman filter paper.

To 5 mL of each sample obtained, 6 M NaCl was added and heated in a water bath at $90\pm 2^\circ\text{C}$ for 90 minutes of protein precipitation. After heating, the samples were centrifuged at 4°C for 10 minutes at 5,000 rounds per minute (rpm) and 1 mL supernatant was pipetted to a test tube and set aside at 4°C , until analysis. Free arginine content was then analysed using the Sakaguchi reaction as follows: To the sub-samples, 0.1% α -naphthol (1 mL), 10% KOH (1 mL), 5% urea (1 mL) and 0.4% K-hypobromite (2 mL) were added [21], the reactions were incubated at room temperature for 20 min and then, measured for absorbance at 520 nm using a spectrophotometer and the results were noted. Concentration of arginine was recorded for each type in triplicate considering the mean. Standard readings were taken using 100, 200, 400, 800, 1600 $\mu\text{g/g}$ arginine concentration commercially available as L-Arginine (GRM038) purchased from Hi Media, Mumbai, India.

STATISTICAL ANALYSIS

Data were entered in Microsoft (MS) Excel and analysed in SPSS version 25.0. Descriptive statistics are presented as mean, SD and

SE. The Shapiro-Wilk test was applied to find normality. ANOVA test was applied to find out any statistical difference between the groups. Tukey's test was used for Post-hoc analysis. The p-value <0.05 was considered as statistically significant.

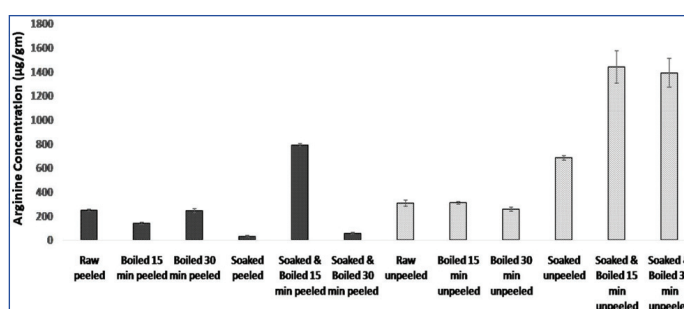
RESULTS

After calculating the mean arginine concentration values, it was found that the raw soaked peeled peanuts had the lowest concentration of arginine (31.82 $\mu\text{g/g}$) and the soaked boiled for 15 minutes unpeeled peanuts had the highest amount of arginine (1438.18 $\mu\text{g/g}$). [Table/Fig-1,2]. Post-hoc tukey's analysis showed that raw peeled (G1), boiled 15 minutes peeled (G2), boiled 30 minutes peeled (G3), soaked peeled (G4), soaked and boiled 30 min peeled (G6), raw unpeeled (G7), boiled 15 min unpeeled (G8), boiled 30 min unpeeled (G9) were having lower concentrations of arginine when compared with soaked and boiled 15 min peeled (G5), soaked unpeeled (G10), soaked and boiled 15 min unpeeled (G11), and soaked and boiled 30 min unpeeled (G12) that was statistically significant with p-value <0.001 . The significantly higher amounts of arginine was observed in unpeeled soaked, unpeeled soaked and boiled for 15 minutes and 30 minutes (G10, G11 and G12 groups) [Table/Fig-3].

Groups	Arginine content ($\mu\text{g/g}$ seed)	Standard Error (SE)	Standard Deviation (SD)
G1: Raw peeled	246.97	8.24	14.25
G2: Boiled 15 min peeled	138.24	7.28	12.59
G3:Boiled 30 min peeled	245.50	16.79	29.04
G4: Soaked peeled	31.82	5.71	9.87
G5: Soaked and boiled 15 min peeled	790.33	9.65	16.69
G6: Soaked and boiled 30 min peeled	56.72	6.13	10.60
G7: Raw unpeeled	304.78	25.03	43.30
G8: Boiled 15 min unpeeled	310.13	10.64	18.40
G9: Boiled 30 min unpeeled	255.37	18.13	31.36
G10: Soaked unpeeled	682.53	18.78	32.48
G11: Soaked and boiled 15 min unpeeled	1438.18	133.80	231.47
G12: Soaked and boiled 30 min unpeeled	1389.47	119.59	206.89

[Table/Fig-1]: Arginine concentration in various groups of peanuts.

ANOVA test F-value=132.73, p-value <0.001 ; p-value was found to be highly significant



[Table/Fig-2]: Arginine concentration in various groups (12) of peanuts estimated spectrophotometrically and concentrations represented as mean values ($\mu\text{g/g}\pm\text{SE}$).

Comparative analysis of all groups	G1	G2	G3	G4	G5	G6	G7	G8	G9	G10	G11	G12
G1	-	0.965	1	0.021	<0.001	0.999	0.995	0.986	1.000	<0.001	<0.001	<0.001
G2	0.965	-	0.956	0.466	<0.001	0.558	0.395	0.314	0.909	<0.001	<0.001	<0.001
G3	1.000	0.956	-	0.019	<0.001	1.000	0.996	0.990	1.000	<0.001	<0.001	<0.001
G4	0.021	0.466	0.019	-	<0.001	0.002	0.001	<0.001	0.011	<0.001	<0.001	<0.001
G5	<0.001	<0.001	<0.001	<0.001	-	<0.001	<0.001	<0.001	<0.001	0.009	<0.001	<0.001
G6	0.999	0.558	1.000	0.002	<0.001	-	1.000	1.000	1.000	0.001	<0.001	<0.001

G7	0.995	0.395	0.996	0.001	<0.001	1.000	-	1.000	0.999	0.001	<0.001	<0.001
G8	0.986	0.314	0.990	<0.001	<0.001	1.000	1.000	-	0.997	0.002	<0.001	<0.001
G9	1.000	0.909	1.000	0.011	<0.001	1.000	0.999	0.997	-	<0.001	<0.001	<0.001
G10	<0.001	<0.001	<0.001	<0.001	0.009	0.001	0.001	0.002	<0.001	-	<0.001	<0.001
G11	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	-	0.887
G12	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.887	-

[Table/Fig-3]: Post-hoc tests for comparative analysis of significance between the tested groups.

p<0.05 statistically significant; Bold p-values are significant

DISCUSSION

As there are numerous benefits and uses of arginine in disease recovery, the present study was done to estimate the arginine composition in the grouped peanuts as peeled or unpeeled, soaked or boiled, soaked and boiled varieties that could be added as a supportive adjuvant therapy in patients. The present study revealed that soaked and boiled peanuts were found to have significantly increased amount of arginine than the raw, soaked varieties. The unpeeled variety of peanuts have shown a remarkable amount of arginine (1438.18 µg/g) than the peeled peanuts.

Adeyeye EI [22] studied the effect of cooking and roasting on amino acids. Arginine content was found to be more in cooked seeds compared to raw and roasted seeds. The results of the present study are in concurrence with this author. The peanuts that were soaked and boiled for 15 minutes had the highest amount of arginine. But, there was a slight decrease in arginine when the boiling was extended to 30 minutes. A study from South East Nigeria revealed that arginine content was 6.89 g/100 g in raw seeds, but, it increased to 6.97 g/100 g in cooked seeds. [23]. In the present study, the raw seeds had lower content of arginine, when compared with soaked and boiled group of peanuts.

In a research performed by Nuzhat H [24], on legumes to study the effect of soaking and cooking on nutritional quality and safety of legumes, they concluded that there was a significant reduction in anti-nutrient substances like phytic acid and tannins and also there was a slight decrease in the protein, minerals and total sugars. This was also observed in the present study that boiling till 30 minutes had significantly lower content of arginine when compared with boiling for 15 minutes. There is very little literature available regarding the nutritional variation on peeled and unpeeled varieties of peanuts. The present study reveals that, there was a lower value of arginine in peeled peanuts than unpeeled peanuts. Further research is recommended to know the exact role of peel on nutritional content.

There are other seed sources for arginine like sunflower, sesame and pumpkin seeds [25] but, the availability of these seeds is not perennial and are little costlier. It was observed that peanuts contain 1 g of arginine per 30 g of biomass and are affordable and readily available worldwide [26]. The free radical scavenging property of L-Arginine inhibits the activity of pro-oxidant enzymes and thus, acts as an antioxidant and these roles of L-Arginine are mediated by Nitric Oxide (NO) [27] that, may aid in early recovery of diseases.

Limitation(s)

The manual extraction of arginine from peanuts might have resulted in lowering the arginine content, whereas automated methods may be implemented to reduce the error.

CONCLUSION(S)

The study concludes that peanuts that were soaked, boiled and unpeeled had the highest arginine content, which could be chosen as adjuvant nutritional therapeutic supplement to aid in the recovery of certain diseases. The availability of literature regarding the use

of arginine containing nuts and seeds was very minimal, therefore further research is recommended.

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